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Evolutionary diversification in stickleback affects ecosystem functioning

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Explaining the ecological causes of evolutionary diversification is a major focus of biology, but surprisingly little has been said about the effects of evolutionary diversification on ecosystems[1–3]. The number of species in an ecosystem and their traits are key predictors of many ecosystem-level processes, such as rates of productivity, biomass sequestration and decomposition[4,5]. Here we demonstrate short-term ecosystem-level effects of adaptive radiation in the threespine stickleback (*Gasterosteus aculeatus*) over the past 10,000 years. These fish have undergone recent parallel diversification in several lakes in coastal British Columbia, resulting in the formation of two specialized species (benthic and limnetic) from a generalist ancestor[6]. Using a mesocosm experiment, we demonstrate that this diversification has strong effects on ecosystems, affecting prey community structure, total primary production, and the nature of dissolved organic materials that regulate the spectral properties of light transmission in the system. However, these ecosystem effects do not simply increase in their relative strength with increasing specialization and species richness; instead, they reflect the complex and indirect consequences of ecosystem engineering by sticklebacks. It is well known that ecological factors influence adaptive radiation[7,8]. We demonstrate that adaptive radiation, even over short timescales, can have profound effects on ecosystems.

Most previous studies of adaptive radiation have focused on the effects of environmental factors on diversification. For example, decades of work in 'model' systems of adaptive radiation, like Caribbean *Anolis* lizards[9], Galapagos finches[10] and African cichlids[8], have revealed how species' environments shape speciation and adaptation. Ecological theory suggests that diversification of one into many species, each with distinct environmental roles, can affect prey diversity, energy flow, food chain length and other aspects of ecosystems[5,7,8,11,12]. Adaptive diversification leading to greater predator diversity, for example, could increase top-down control over a wider range of consumer species and strengthen cascading effects of predators on lower trophic levels[13,14]. It follows that adaptive radiation may modify the environmental conditions of ecosystems and shape the selective pressures of other species[12,15]. However, in general, the consequences of evolutionary diversification for ecosystems are largely unknown.

We introduced threespine stickleback into outdoor mesocosms to study the short-term effect of speciation and specialization of stickleback on pond ecosystems. These fish are common in marine coastal waters of the Northern Hemisphere, and have colonized and adapted to freshwater habitats in coastal areas[16]. In seven lakes of British Columbia, sympatric species pairs occupy different niches within the same lake: one limnetic and the other benthic. These two forms have evolved independently multiple times in the region over the past 10,000 years[17], they differ by morphology and habitat use, and they are reproductively isolated with low levels of gene flow[18]. Most relevant to their effects on ecosystems, the two forms have distinct diets: limnetic and benthic forms specialize on pelagic zooplankton and littoral invertebrates, respectively. In contrast, sticklebacks that have not diversified, and occur alone in similar small lakes, have an intermediate form and a more generalist diet[6,7] (see Supplementary Fig. 1). Previous studies have found that sticklebacks can cause trophic cascades in pond ecosystems[19]. Here we address whether the evolutionary diversification of stickleback populations affects ecosystem function.

We conducted an experiment to test the effects of evolutionary diversification on ecosystems using benthic and limnetic fish from Paxton Lake—one of the seven lakes with a sympatric species pair[17]—and an intermediate generalist fish from a nearby single-species lake, Cranby Lake. We introduced the fish into mesocosms (1,136 l) according to four treatments, each replicated ten times: generalist (G; Cranby Lake fish), limnetic (L), benthic (B), and species pair (LB; all from Paxton Lake). Treatments G and BL represent combinations that occur in natural lakes, whereas the B and L treatments were used to evaluate the effects of specialization independent from an increase in species richness. Each mesocosm initially contained the same total biomass of fish (between 5.0 and 6.0 g); because stickleback forms differ in body size, each tank contained between three and six fish ($G = 4$, $B = 3$, $L = 6$, $BL = 5$). Mesocosms contained both benthic and pelagic zones and were seeded with sediments and invertebrates from nearby ponds inhabited by sticklebacks. Because predators can influence ecosystems through both trophic and non-trophic interactions[20,21], we examined whether stickleback diversification could influence a wide variety of ecosystem properties and functions over a 10-week period (see Supplementary Methods for details). We evaluated differences in these measures among treatments, with a focus on overall effects and two sets of planned contrasts: between G and BL, to test the overall effects of evolutionary diversification, and between BL and either B or L, to test for the effects of diversification versus specialization alone.

We tested two possible hypotheses for the consequences of stickleback diversification on aquatic food webs and ecosystems (see Supplementary Fig. 1). Our first hypothesis, arising from trophic cascade theory (H1), was that sticklebacks would cause the strongest cascades in their preferred foraging habitats, leading to higher levels of primary productivity with increasing predator specialization ($G < B,L < BL$). Our second hypothesis was that distinct types of sticklebacks might differ in their engineering effects on ecosystems (H2).

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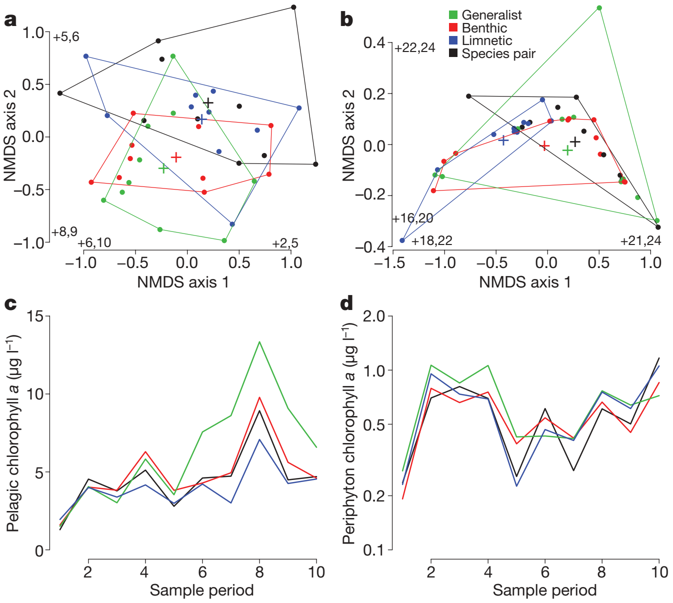

Figure 1 | Differences in community structure and productivity among treatments. a, **b**, First two non-metric multidimensional scaling (NMDS) axes of pelagic (**a**) and benthic (**b**) invertebrate biomass. Points represent individual mesocosms, coloured by treatments, crosses represent treatment means, and polygons surround all points for a given treatment. Numbers on each axis correspond to the two species with the strongest positive and negative loadings (see Table 1). **c**, **d**, Amount of chlorophyll in pelagic (phytoplankton, **c**) and benthic (periphyton, **d**) zones at ten sampling periods through the course of the experiment. Coloured lines represent treatment means weekly throughout the experiment.

Sticklebacks are part of a complex food web and could affect ecosystems by a whole suite of trophic and non-trophic interactions, including indirect mechanisms such as habitat modification and nutrient recycling[22]. In particular, on the basis of previous research on ecosystem engineering by fish[23,24], we hypothesized that sticklebacks could affect light transmission in the water column by affecting algal biomass through a combination of cascading trophic interactions and non-trophic engineering interactions (for example, nutrient recycling, habitat modification). Fish can affect light transmission in the water column by indirectly influencing the abundance of suspended algal particles which both directly block light[24] and produce dissolved organic substances that attenuate light[25] (see Supplementary Fig. 1). Dissolved organic carbon (DOC) produced by algae, compared to that released from the decomposition of leaf litter and sediment organic matter, is more easily degradable by bacteria and more transparent to light in the ultraviolet range[26]. As such, our second hypothesis was that sticklebacks would have indirect effects on algae, DOC and the light transmission properties of the water; however, we made no specific predictions about whether diversification or specialization of fish would have larger ecosystem-engineering effects.

We found that stickleback diversification affected the community composition of organisms at lower trophic levels. We found strong effects of sticklebacks on pelagic prey communities (Fig. 1a; multivariate analysis of variance (MANOVA) on two-dimensional non-metric multidimensional scaling (NMDS) using zooplankton species' total biomass per tank, Wilks' $\lambda = 0.65$, $P = 0.02$), with a significant contrast between treatments representing the effects of stickleback diversification (G versus BL, Hotelling $T^2 = 10.9$, $P = 0.02$). Contrasts comparing the effects of specialization versus diversification attribute this effect to specialization alone and, specifically, to the transition from the generalist to the limnetic form. Pelagic prey communities with two specialists were significantly different compared with those with only the benthic form (B versus BL, NMDS Euclidean distance 0.60, $T^2 = 9.9$, $P = 0.02$) but not from those with only the limnetic form (L versus BL, NMDS Euclidean distance 0.17, $T^2 = 0.7$, $P = 0.7$). The calanoid copepod (*Skistodiaptomus oregonensis*) was virtually eliminated from tanks with limnetics (that is, both the L and LB treatments, Table 1). In natural lakes, this zooplankton species is highly transparent and vertically migrates through the water column to avoid stickleback predation, but, nevertheless, it is a common diet item for both limnetics and pelagic specialists[27]. Hence, predator specialization over the course of an

Table 1 | Species, average lengths and average densities (per litre) for pelagic and benthic organisms at the end of the experiment

	Size (mm)	Treatment			
		Generalist (G)	Benthic (B)	Limnetic (L)	Species pair (BL)
Pelagic					
(1) *Alonella*	0.25	678	625	337	862
(2) *Bosmina*	0.24	16	222	58	23
(3) *Chaoborus*	5.33	0	0	4	0
(4) Chironominae	0.86	33	67	49	14
(5) *Chydorus sphaericus*	0.24	120	119	144	444
(6) *Daphnia*	0.88	0	5	16	0
(7) *Diaphanosoma*	0.36	5	57	4	9
(8) *Sida crystallina*	0.53	0	5	0	0
(9) *Skistodiaptomus oregonensis*	0.73	552	351	53	14
(10) Cyclopoida	0.47	93	248	428	680
(11) Mite	0.37	0	0	4	0
(12) Nauplii	0.17	9,787	10,093	7,210	7,102
(13) Stone fly larva	0.98	0	26	0	0
(14) Surface insect	3.21	0	5	0	0
Benthic					
(15) Amphipoda	3.68	0	0	0	1
(16) Chironomidae	3.9	255	237	140	284
(17) Cladocera	1.1	2	7	4	26
(18) Coleoptera	2.76	0	1	1	0
(19) Diptera	3.35	0	0	0	1
(20) Gastropoda	2.02	2	9	10	5
(21) Hydracarina	0.26	0	1	1	1
(22) Odonata	7.7	2	0	0	1
(23) Ostracoda	0.52	0	104	10	28
(24) Pelecypoda	3.2	0	0	0	3
(25) Trichoptera pupae	5.35	0	0	7	0
(26) Other pupae	3.54	0	2	0	3

Table 2 | Results of profile analysis on measures of productivity and respiration

Test	Comparison	Test statistic	Phytoplankton	Periphyton	NPP	GPP	Respiration
Time	Overall	$F_{28,9}$	34.9§	99.9§	258.3§	228.7§	439.5§
	G versus BL	$F_{12,9}$	7.9‡	19.2§	59.3§	51.8§	93.9§
	B versus BL	$F_{12,9}$	9.8§	20.1§	49.1§	46.2§	96.5§
	L versus BL	$F_{12,9}$	6.5‡	21.2§	55.3§	50.0§	96.3§
Treatment	Overall	$F_{3,36}$	3.1†	0.3	4.4‡	5.8‡	6.3‡
	G versus BL	$F_{1,18}$	3.8*	0.7	2.5	4.7†	8.0†
	B versus BL	$F_{1,18}$	0.1	0.1	3.2*	3.0*	1.9
	L versus BL	$F_{1,18}$	0.7	0.0	0.1	0	0.1
Time by treatment interaction	Overall	Wilks' λ	0.29*	0.39	0.31*	0.38	0.48
	G versus BL	T^2	38.9*	16.7	55.5†	21.2	24.9
	B versus BL	T^2	9.4	28.9	5.5	5.1	16.9
	L versus BL	T^2	12.6	22.2	22.2	24.1	10.7

The table entries represent values of test statistics. See Supplementary Information for details of the profile analysis.
* $P < 0.1$.
† $P < 0.05$.
‡ $P < 0.01$.
§ $P < 0.0001$.

adaptive radiation could potentially alter the strength of trophic interactions between prey and their resources. We found no differences among treatments in the composition of benthic invertebrate communities (Fig. 1b, MANOVA on two-dimensional NMDS using an index of total biomass (see Supplementary Information) for each species in each tank, Wilks' $\lambda = 0.8$, $P = 0.3$).

Treatments differed strongly in their gross primary productivity (GPP), net primary productivity (NPP) and respiration (R = GPP − NPP), with the strongest differences occurring between the G and BL treatments (Table 2). Treatments also differed in their levels of algal biomass (Table 2 and Fig. 1c), again with the strongest differences occurring between the G and BL treatments (Table 2). There were no differences detected between treatments in the amount of benthic algae (periphyton, Table 2 and Fig. 1d). Despite the strong effects of limnetics on pelagic zooplankton community structure, the treatments including this species (L and BL) did not have the highest algal biomass, contrary to our hypothesis based on trophic cascade theory (H1). Instead, the generalist treatment had the most algae (Fig. 1c), implying that neither specialization nor diversification led to stronger trophic cascades. Strong contrasts in the strength of cascades that affect algal biomass are more likely when zooplanktivorous fish vary in their foraging efficiency on predominantly herbivorous (for example, *Daphnia*) rather than omnivorous zooplankton taxa (for example, copepods). In the current experiment, *Daphnia* were reduced to very low abundance by sticklebacks in all treatments. As a result, the zooplankton communities differed primarily in their relative abundance of omnivorous and predation-resistant *Diaptomus* copepods (Table 1)[22]; for this reason, we may not have detected a difference in the strength of trophic cascades among treatments.

To investigate the possible ecosystem engineering effects of sticklebacks we tested whether several ecosystem properties (see Supplementary Information) differed among stickleback treatments. We focused on the composition of DOC because it can profoundly affect the biology and physics of aquatic ecosystems by affecting the transmissibility of different wavelengths of light through the water[25,26,28,29]. In agreement with the second hypothesis, we found that sticklebacks act as ecosystem engineers[23], strongly affecting the composition of the DOC pool and the physical light environment. Treatments were similar in their total amount of dissolved organic carbon (DOC, $F_{3,36} = 0.38$, $P = 0.8$; Fig. 2a), but varied in their DOC composition. By the end of the experiment, water differed significantly among treatments in its transmission of both photosynthetically available radiation (PAR: light with wavelengths between 400 and 700 nm; $F_{3,36} = 3.8$, $P = 0.02$; Fig. 2b) and ultraviolet radiation (A_{320}: $F_{3,36} = 6.3$, $P = 0.001$, Fig. 2c; this result was the same over the entire ultraviolet band (280–400 nm, see Supplementary Information)). Furthermore, dissolved substances fluoresced significantly more at shorter excitation wavelengths in the generalist treatment (Lγ (low-molecular-mass molecules, <5,000 Da, that fluoresce at low excitation wavelengths); ANOVA of Lγ/total $F_{3,33} = 2.9$, $P = 0.05$, no planned contrasts were significant; Fig. 2d). As above, the planned contrasts revealed effects of both diversification and specialization on the light environment in the mesocosms (PAR: diversification, $t = -2.7$, $P = 0.02$, specialization, B versus BL, $t = -1.0$, $P = 0.3$, L versus BL, $t = -2.6$, $P = 0.02$; A_{320}: diversification, $t = -2.92$, $P = 0.009$, specialization, B versus BL, $t = -0.7$, $P = 0.5$, L versus BL, $t = -0.1$, $P = 0.9$). Differences among treatments in light transmission were due to the dissolved substances rather than the algal particles themselves (see Supplementary Information). DOC in the generalist (G) treatment was dominated by low-molecular-mass compounds originating from *in situ* primary production[28], whereas DOC in the benthic (B) treatment was dominated by larger molecules such as fulvic

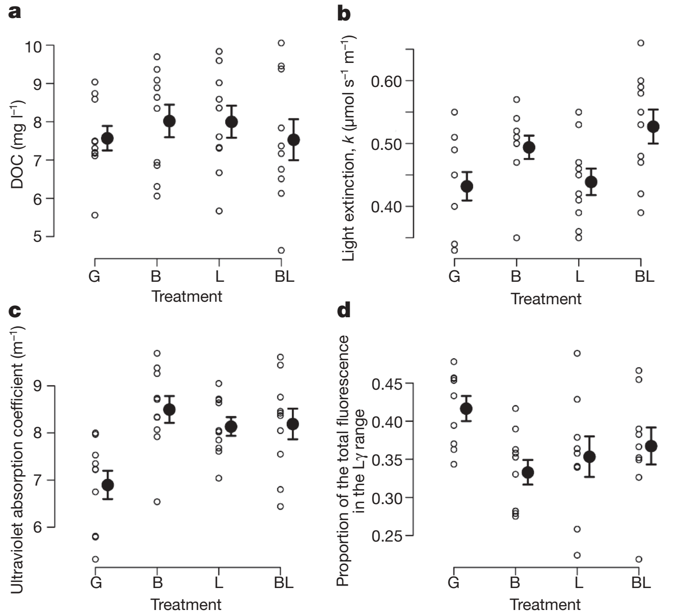

Figure 2 | Dissolved organic content and light transmission across treatments. Small open circles are individual data points; large filled circles are means ± s.e. ($n = 10$). **a**, Concentration of dissolved organic matter (DOC). **b**, Extinction coefficients of photosynthetically available radiation (PAR, 400–700 nm). **c**, Absorption coefficients for ultraviolet light (A_{320} m^{-1}). In **b** and **c**, lower values indicate higher transparency in the given range of wavelengths. **d**, Ratio of low-molecular-mass molecules (Lγ < 5,000 Da, low excitation wavelengths) to higher molecular mass molecules (Mγ and Hγ, higher excitation wavelengths; see Supplementary Information). A high ratio indicates that the dissolved substances in the water have a smaller size distribution.

acids, which are produced by leaf litter decomposition[29] (see Supplementary Information). Because the composition of algal communities is quite sensitive to small changes in the concentration of dissolved substances[30], we believe that the differences in light penetration caused by different stickleback predators is relevant for natural lake environments (see Supplementary Information).

This experiment reveals that both specialization and speciation of predators can affect how ecosystems function. Feedbacks between evolutionary diversification and ecosystems are rarely explored but have implications for understanding adaptive radiations. We know that ecology and environmental conditions affect the selective environment for specialization, divergence and speciation[8]. The effect of organisms on their environment through ecosystem engineering could provide a complementary explanation for the tendency of some groups to radiate explosively[15,23]. Our results illustrate that the effects of stickleback diversification on ecosystem function result from both trophic and non-trophic interactions, and suggest that even short periods of adaptive radiation (~10,000 years) have the potential to transform the structure and functioning of ecosystems. It remains to be seen how the evolution of trophic interactions and ecosystem-engineering effects of predators might shape the evolution of other species in the food web.

METHODS SUMMARY

Mesocosms were constructed from Rubbermaid cattle watering tanks, each 1,136 l, and filled with a mixture of sand and gravel, benthic substrate from natural ponds, well water, and nutrients (Na^+ and PO_4^-; see Supplementary Information for details). We collected fish from lakes on Texada Island, British Columbia, Canada. Most fish were collected, transported and added to tanks at the beginning of the experiment, but some supplemental fish were collected and transported later in the experiment (see Supplementary Information for details). There was some mortality during the experiment (total deaths = 154). Limnetic fish were more likely to die than benthic fish, although fish biomass did not differ among treatments over the course of the experiment. When dead fish were located, they were removed from the tanks and replaced (see Supplementary Information).

We measured prey community composition by sampling species abundance from the pelagic and benthic zones at the end of the experiment (see Supplementary Information for details). We also took several measures of ecosystem function at regular intervals during the course of the experiment, including chlorophyll *a*, total dissolved phosphorus, nitrates, ammonia, net primary productivity (NPP), gross primary productivity (GPP) and decomposition rates. Finally, we used several techniques to measure and describe the DOC in the tanks, including ultraviolet scanning spectroscopy (UVSS) to measure absorption[25], synchronous fluorescence spectroscopy (SFS) to investigate molecular structure[26], and fluorescence excitation-emission matrices (FEEM) to assess the relative concentration and potential origins of organic matter[29]. Full descriptions of all measurements, calculations and data analyses are available in Supplementary Information.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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